

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the Claims

1. (Currently Amended) A method of assembling several DNA units in sequence in a DNA construct, which method comprises the steps of

a) providing each DNA unit with a restriction enzyme recognition sequence at its 5' end and with a recognition sequence for the same restriction enzyme at its 3' end, said 3' recognition sequence also comprising a DNA modification enzyme recognition sequence ~~that is combined with a recognition site for a DNA modification enzyme~~

b) providing a starting DNA construct having an accessible restriction site for the same or a compatible restriction enzyme and cleaving the starting DNA construct with such a restriction enzyme,

c) inserting a the desired DNA unit into the DNA construct, thereby generating a ligated product, and bringing the ligated product into contact with a DNA modification enzyme such that the restriction site at the 3' end of the desired ~~inserted~~ DNA unit is abolished,

d) cleaving the ligated product at an accessible unmodified recognition site for the same or a compatible restriction enzyme,

e) repeating steps c) and d) to introduce each subsequent desired DNA unit to give a DNA construct containing all the desired units in sequence.

2. (Previously Presented) The method of claim 1 wherein the DNA modification enzyme is a methylase.

3. (Currently Amended) The method of claim 2 wherein the methylase is a the dam methylase of *Escherichia coli*.

4. (Currently Amended) A method of assembling several DNA units in a DNA construct which method comprises the steps of

- a) providing each DNA unit with a XbaI ~~an XbaI~~ recognition sequence 5'XXTCTAGA3' (where XX is not GA) at its 5' end and with a XbaI ~~an XbaI~~ recognition sequence 5'GATCTAGA3' at its 3' end,
- b) providing a starting DNA construct having an accessible XbaI ~~XbaI~~ site and cleaving the starting DNA construct with XbaI ~~XbaI~~,
- c) inserting the desired DNA unit to the DNA construct, thereby generating a ligated product, and using the a resulting ligated product to transform a *dam*⁺ strain of *E. coli*,
- d) recovering the a resulting- ligated product plasmid and cleaving the ligated product plasmid at an accessible XbaI ~~XbaI~~ site with XbaI ~~XbaI~~,
- e) repeating steps c) and d) to introduce each subsequent desired DNA unit to give a DNA construct containing all the desired units in sequence.

5. (Previously Presented) The method of any one of claims 1 to 3, wherein the recognition sequences for the restriction enzyme and the DNA modification enzyme are created in the DNA units prior to cutting with the restriction enzyme.

6. (Currently Amended) The method of any one of claims 1 to 4, ~~claims 1 to 4~~ wherein the restriction sites are created in each DNA unit ~~the fragment~~ by means of a primer extension reaction.

7. (Previously Presented) The method of any one of claims 1 to 4, wherein the DNA construct is an expression vector capable of facilitating expression of the protein encoded by the desired DNA units.

8. (Currently Amended) The method of claim 3, wherein the DNA modification is removed and the restriction site re-established by replicating the ligated product in a *dam*⁻ strain of *E. coli* ~~by means of a suitable vector~~.

9. (Currently Amended) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

- a) providing a starting DNA construct comprising a first DNA unit with a recognition sequence for a first restriction enzyme at the its 3' end of said DNA unit, and cleaving the said first DNA unit ~~ie~~ with said first restriction enzyme,
- b) providing a desired ~~each other~~ DNA unit with a recognition sequence at its 5' end for a second restriction enzyme which has a compatible ligation sequence with that of the first restriction enzyme, and a downstream recognition sequence for said first restriction enzyme followed by a downstream recognition sequence for a third restriction enzyme at its 3' end, and cleaving ~~each~~ said other desired DNA unit with the second and third restriction enzymes,
- c) ligating the said starting DNA construct ~~first DNA unit~~ with ~~the~~ a desired ~~other~~ DNA unit to form a ligated product such that the ligation of the starting DNA construct and the desired DNA unit ~~two units~~ abolishes the recognition site for the first restriction enzyme at the ligation junction, and cleaving the ligated product with said first restriction enzyme,
- d) repeating step b) with a subsequent desired DNA unit and ligating said subsequent desired DNA unit with the product from c) ~~with a desired DNA unit from b)~~ to form a ligated product and cleaving the ligated product with said first restriction enzyme
- e) repeating step d) with each desired ~~other~~ DNA unit in turn so as to assemble the DNA units in sequence.

10. (Currently Amended) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

- a) providing a starting DNA construct comprising a first DNA unit with a XbaI an ~~XbaI~~ recognition sequence 5'TCTAGA3' at its 3' end, and cleaving the said first DNA unit with XbaI ~~XbaI~~,
- b) providing a desired ~~each other~~ DNA unit with a SpeI ~~SpeI~~ recognition sequence 5'ACTAGT3' at its 5' end, and downstream XbaI ~~XbaI~~ recognition sequence 5'TCTAGA3' followed by a downstream SmaI ~~SmaI~~ recognition sequence 5'CCCGGG3' at its 3' end, cleaving ~~each~~ said other desired DNA unit with SpeI ~~SpeI~~ and SmaI ~~SmaI~~, and dephosphorylating the 5' end of the cleaved DNA unit,
- c) ligating the said starting DNA construct ~~first DNA unit~~ with ~~the~~ a desired ~~other~~ DNA unit to form a ligated product and cleaving the ligated product with XbaI ~~XbaI~~,

d) repeating step b) with a subsequent desired DNA unit and ligating said subsequent desired DNA unit with ligating the product from c) ~~with a desired DNA unit from b)~~ to form a ligated product and cleaving the ligated product with XbaI-XbaI

e) repeating step d) with each desired ~~other~~ DNA unit in turn so as to assemble the DNA units in sequence.

11. (Previously Presented) The method of claim 9 or claim 10 wherein the assembly occurs *via* stepwise addition of at least one DNA unit to a vector.

12. (Previously Presented) The method of claim 9 or claim 10 wherein the said first DNA unit is attached to the solid phase for use in step c).

13. (Currently Amended) The method of claim 12, wherein the solid phase is combined with a subsequent desired DNA unit in step c) ~~split and mixed between steps c), d), and e)~~ to make several different assemblies.

14. (Previously Presented) The method of claim 9 or claim 10, wherein the recognition sequences in one or more of the DNA units are introduced by means of extension primers.

15. (Currently Amended) The method of claim 9 or claim 10, wherein the assembly of several DNA units is inserted into ~~in to~~ an expression vector which is used to transform a host capable of expressing the protein encoded by the assembly of several DNA units ~~vector~~.

16. (Previously Presented) The method of any one of claims 1, 4, 9, or 10, wherein one or more of the DNA units encodes a catalytic or transport protein domain.

17. (Currently Amended) The method of claim 16 wherein one or more of the DNA units are derived from DNA sequences of polyketide synthesising enzyme domains ~~domain~~ DNA sequences.

18. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from peptide synthesising enzyme domain DNA sequences.

19. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from hybrid peptide polyketide enzyme domain DNA sequences.

20. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from fatty acid synthesizing enzyme domain DNA sequences.

21. (Previously Presented) The method of claim 16 wherein one or more of the DNA units encode modules comprising one or more catalytic or transport domains.

22.-48. (Canceled)